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14. ABSTRACT

Epithelial ovarian cancer (EOC) continues to be the leading cause of the death among gynecological malignancies, owing to the lack of preventive strategies, early diagnostic methods or effective therapies. Detailed understanding of molecular changes, such as, somatic mutations, in ovarian cancer holds the promise of greatly contributing to the understanding of ovarian cancer pathogenesis, with obvious implications in development of new biomarkers, prevention strategies and therapyMicro-RNAs (miRNAs) are endogenous non-coding ~22 nucleotide (nt) RNAs, whose expression appears to be elevated in normal tissues, compared to in tumors, suggesting that silencing of miRNA may be a hallmark of human cancers. MiRNA misexpression might be due to genetic mutations in miRNA genes and their responsive elements in target genes. To test this hypothesis, In the past year, we identified 7 genetic mutations in 50 selected human miRNA genes and their responsive elements in target genes in 75 OC tumor tissues. Their correlations with clinical outcome under investigation.

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INTRODUCTION

Epithelial ovarian cancer (EOC) continues to be the leading cause of the death among gynecological malignancies, owing to the lack of preventive strategies, early diagnostic methods or effective therapies. Detailed understanding of molecular changes, such as, somatic mutations, in ovarian cancer holds the promise of greatly contributing to the understanding of ovarian cancer pathogenesis, with obvious implications in development of new biomarkers, prevention strategies and therapy. It is our hope that this investigation will yield signatures of somatic mutations that will help us predict disease outcome and possibly address crucial clinical management issues, such as identification of patients what will respond to standard chemotherapy, in need for alternate frontline drugs. Micro-RNAs (miRNAs) are endogenous non-coding ~22 nucleotide (nt) RNAs, whose expression appears to be elevated in normal tissues, compared to in tumors, suggesting that silencing of miRNA may be a hallmark of human cancers. MiRNA misexpression might be due to genetic mutations in miRNA genes and their responsive elements in target genes. To test this hypothesis, we plan to identify genetic mutations in selected human miRNA genes and their responsive elements in target genes in 75 OC tumor tissues and correlate somatic mutations in miRNA genes and their responsive elements in target genes with poor clinical outcome in EOC.

BODY

In the past one year, we have successfully sequenced 50 microRNAs in 75 OC tumor tissues. So far, seven novel somatic mutations were observed in seven primary or precursor miRNA genes.

Table. Somatic mutations in selected miRNA genes in ovarian tumor tissues

miRNA genes	Nt	Location	MAF
Novel variants:			
miR-199	G/C	Pri-miRNA	0.036
miR-191	A/T	Pri-miRNA	0.018
miR-29b-2	T/C	Pri-miRNA	0.018
miR-17	A del	Pri-miRNA	0.036
miR-92	A/T	Pri-miRNA	0.009
miR-26a	C/T	Pri-miRNA	0.036
miR-188	G/A	Pri-miRNA	0.045

We will continue to perform sequencing analysis in more microRNAs in the next 6 months.

It is hypothesized that the presence of genetic variants in pri- or pre-miRNA, but not within the mature miRNA itself, could affect their secondary structure and thereby block processing into functional mature miRNA. To test this hypothesis, we used RNAHybrid to predict and calculate the most stable second ary RNA structure with the lowest free energy for the variant and the wildtype *pre-hsa-miR-188*. From the predicted secondary structures, a structure change was observed in the *pre-hsa-miR-188* A allele compared to

the G allele (Figure). The G allele created a base-pairing, which strengthen the stability of the stem and changes the secondary structure of this pre-miRNA. The optimal free energy was increased from -39.20 Kcal/Mol for A allele to -41.60 kcal/Mol for G allele, suggesting a more stable secondary structure for G allele than A allele. Processing of miRNA precursors by the RNase Drosha requires the secondary hairpin structure characteristic of these RNA molecules and specific sequence elements within the pri-miRNA. To assess the effect of the predicted changes in secondary structure on mature miRNA expression, we cloned a wild-type or variant *hsa-miR-188* miRNA gene into the pcDNA3.1 expression plasmid (Invitrogen, CA) and transfected the constructs into PC-3 prostate cancer cell line. The expression levels of mature miRNAs were measured by Taqman based microRNA assays (Applied Biosystems). We found that the expression levels of mature hsa-miR-188 in the variant miRNA gene were over 3 times higher than those in wild-type. Using the PITA prediction algorithm, *MLH1 and MSH2* are predicted as targets for *hsa-miR-188*. Therefore, our results suggest that a functional somatic mutation in the *pre-miR-188* gene might alter the expression of mature miRNA and, thereby, contribute to ovarian tumor development and cancer progression through regulating key ovarian cancer related genes.

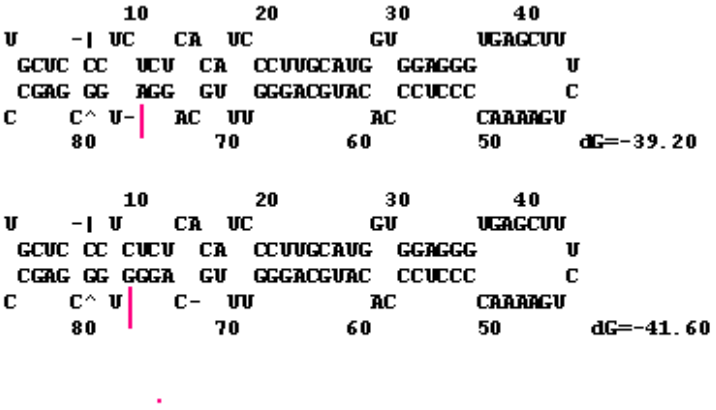

Figure5 . Secondary structure of miR-188 A and G alleles

KEY RESEARCH ACCOMPLISHMENTS

We have successfully sequenced 50 microRNAs in 75 OC tumor tissues. So far, seven novel somatic mutations were observed in seven primary or precursor miRNA genes.

WE found a functional somatic mutation in miR-188. This mutation could alter the expression of mature miRNA and, thereby, contribute to ovarian tumor development and cancer progression through regulating key ovarian cancer related genes.

REPORT OUTCOME

We are in the preparation of a manuscript to report our findings.

CONCLUSIONS

So far, the study moves smoothly. We don't expect any problems at current stage. We expect to begin data analysis on the correlations between somatic mutations and clinical outcome soon.